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Processes for the production of noodles by machines

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ABSTRACT

A process for the production of noodles by machines, which comprises the steps of:

(i) preparing a noodle dough with 38 to 55% of water added to a raw material,

(ii) forming the noodle dough into a noodle sheet,

(iii) maturing the noodle sheet at a temperature of 30 to 35°C and a relative humidity of 70 to 90% for 10 to 60 minutes,

(iv) rolling a matured noodle sheet followed by cutting into strands of noodles, and

(v) stretching the strands at a stretching ratio of 1.5 to 2 times into desired strands of a noodle product. The process comprises further the step of adding an edible oil and lipase to the noodle dough. Those processes can produce simply the noodles of high quality having good eating quality in a short time period with good productivity.

(iii) maturing the noodle sheet at a temperature of 30 to 35°C and a relative humidity of 70 to 90% for 10 to 60 minutes,

(iv) rolling a matured noodle sheet followed by cutting into strands of noodles, and

(v) stretching the strands at a stretching ratio of 1.5 to 2 times into a desired strand of a noodle product.

The processes of the present invention will be fully described below in the order of the process steps.

Preparation of Noodle Dough

Raw materials for noodles are principally wheat flour, but the nature of wheat flour required for noodle types is largely different. In addition, grain flour conventionally used according to noodle types can be used, e.g. buckwheat flour, rice flour, soybean flour and starch. In general, it is preferred that wheat flour is used alone or in combination with small amounts of other grain flour. In the present invention, the type of raw materials and their formulation are not particularly limited.

In the preparation of the noodle dough, water is added to raw materials, but a solution of salt is used for the dough of "Udon" (standard noodle) and "Kansui" (a solution containing a mixture of sodium carbonate, potassium carbonate and sodium phosphate) or "Kanpun" (the form of "Kansui" being powdered) are used for the dough of "Ra-men" (Chinese type noodle). When salt is used, the quantity of

by weight of salt to 100 parts by weight of raw materials, the percent of water added is calculated as 45% = [(50 x 0.9)/100] x 100.

In the preparation of the noodle dough, edible oils and lipase may be further added, by which the product can be produced with better viscoelasticity and higher quality. In this case, the use of edible oil alone cannot provide an improvement in viscoelasticity. Therefore, a combined use with lipase is required.

Any edible oil can be used if being liquid at an ordinary temperature, but vegetable oils are preferable. The vegetable oils include salad, sesame, rape, cottonseed and soybean oils. They may be used in one or more combinations. Any lipase of food class can be used.

Preferably, 0.1-1.0 g of edible oils and 250-10,000 units of lipase are added to 100 g of raw materials. More preferably, 0.2-0.6 g of edible oils and 1,000-5,000 units of lipase are added. An activity for liberating an acid corresponding to 1 μmol of oleic acid under a predetermined condition is defined as 1 unit. This unit is determined by emulsifying 100 ml of olive oil and 150 ml of 2% polyvinyl alcohol solution to prepare a substrate, mixing 5 ml of the substrate, 4 ml of McIlvaine's buffer solution (pH 7.0) and 1 ml of 5 x 10^{-4}% enzyme solution to react the mixture at 37°C for 60 minutes, ceasing the reaction after 60 minutes and titrating the reaction

conveyors are basically constructed so that the rotation speed of the belt conveyor B is faster than that of the belt conveyor A and the rotation speed of the belt conveyor C is faster than that of the belt conveyor B. At the positions where noodle strands 35 are going down to the lower belt conveyors B and C, a tension detection roller 37 is pivoted by a lever 38 to a bearing portion 40 which is mounted on an angle member 39 fixed on the casing 36, so that the tension detection roller 37 changes the position through noodle strands 35. At the bearing portion 40 is arranged a controller 41 for a motor (not shown) for driving the belt conveyors. The controller 41 is devised to change the output signal in accordance with a pivot angle of the lever 38.

The noodles which were formed into desired strands according to the stretching treatment of the present invention can be distributed and sold in the form of uncooked wet noodles, dried or semi-dried noodles which were further subjected to drying treatment, boiled and steamed noodles which were further subjected to gelatinization, and frozen or instant noodles which were further subjected to freezing or instant treatment. In any case, the dimension (thickness, width) of noodle strands just after cutting can be reduced gradually by the stretching treatment according to the present invention and formed into the final products such as "Udon", "Hiyamugi", "So-men" and "Ra-men". The processes of the present invention are particularly suitable for the manufacture of dried noodles. The dried noodles can

Table 3 indicates that very thin noodles, "So-men" produced by the processes of Examples 7-12 satisfying the requirements of the present invention for the percent of water added, maturing conditions of noodle sheet and stretching ratio of noodle strands have good eating quality, as compared with those obtained in Comparative Examples 10-17 lacking at least one of the above requirements of the present invention. For the cases of Examples 7-12, only one maturing treatment (maturation of noodle sheet) can produce easily "So-men" having good eating quality, in short time period, with good productivity, as compared with the case of Comparative Example 18 requiring the maturation of noodle strands three times.

Examples 13-18

Very thin dried noodles (dried "So-men") were manufactured in the same manner as in Examples 7-12, but adding 150 g of salad oil (0.6% added to wheat flour) and 50,000 units/kg wheat flour of lipase (available from Nagase Biochemicals Ltd. under the registered trade mark of "LILIPASE" A-10) to the mixture of 25 kg of the same soft wheat flour of medium protein content and 12.5 kg of a saline solution (10% salt concentration) in the preparation of the noodle dough in Examples 7-12.

Immediately after the manufacture, 100 g of the resultant dried "So-men" were boiled in 1 liter of boiling water to a ready-to-eat state, thus giving 280% of a yield of boiled noodles.

The quality of the boiled noodles was assessed by ten trained panelists in accordance with the score shown in Table 1. The averaged results are shown in Table 4.

Comparative Examples 19-26

Very thin dried noodles (dried "So-men") were prepared by using the same raw materials and apparatus as used in Examples 13-18 and further using the varying requirements for the percent of water added, maturing conditions, reduction ratio after maturing and stretching ratio of noodle strands shown in Table 4. The resultant noodles were assessed in the same manner as in Examples 13-18. The averaged results are shown in Table 4.

Table 4

Example No.	Percent of water added (%)	Addition of edible oil	Addition of lipase	Thickness of noodle sheet on maturing (mm)	Maturing conditions Temp. (°C)	Humid. (%RH)	Time (min.)	Frequency	Reduction ratio after maturing (A/B)	Stretching ratio of noodle strands (times)	Noodle quality
Example 13	45	Yes	Yes	4	30	70	30	1	3.1	1.9	4.6
14	45	Yes	Yes	4	35	80	10	1	3.1	1.8	4.6
15	45	Yes	Yes	12	30	90	60	1	9.2	1.6	4.5
16	45	Yes	Yes	2	30	80	30	1	1.5	1.7	4.5
17	38	Yes	Yes	4	35	85	60	1	3.1	1.6	4.5
18	55	Yes	Yes	4	30	70	10	1	3.1	1.9	4.5
Comparative Example 19	49	Yes	Yes	15	35	85	60	1	11.5	1.2	3.2
20	45	Yes	Yes	4	25	90	60	1	3.1	1.1	3.0
21	45	Yes	Yes	4	35	65	60	1	3.1	1.3	3.4
22	45	Yes	Yes	1.3	35	85	60	1	Not reduced	1.2	3.1
23	35	Yes	Yes	4	30	70	30	1	3.1	1.6	3.3
24	58	Yes	Yes	4	30	70	30	1	3.1	1.8	3.2
25	45	Yes	Yes	4	30	70	5	1	3.1	1.4	3.1
26	45	Yes	Yes	4	30	70	70	1	3.1	2.0	3.2

Table 4 indicates that very thin noodles, "So-men" produced by the processes of Examples 13-18 satisfying the requirements of the present invention for the percent of water added, the maturing condition of noodle sheet and the stretching ratio of needle strands and further adding an edible oil and lipase to the noodle dough, have very good eating quality as compared with those obtained in Comparative Examples 19-26 lacking at least one of the above requirements of the present invention. As shown in Comparative Examples 19-26, the process lacking at least one of the above requirements of the present invention cannot provide an improvement over the noodles produced by the processes of the present invention, even if an edible oil and lipase were added.

Advantages of the present invention

According to the process of the present invention, only one maturation (maturation of noodle sheet) can produce simply the noodles of high quality having good eating quality in a short time period with good productivity. Further, the addition of an edible oil and lipase in the preparation of noodle dough can produce the noodles having higher viscoelasticity and better eating quality.

COMMONWEALTH OF AUSTRALIA

No. 570720

LETTERS PATENT

STANDARD PATENT

ELIZABETH THE SECOND, by the Grace of God Queen of Australia and Her other Realms and Territories, Head of the Commonwealth.

To all to whom these presents shall come Greeting:

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Title of Invention: LIPASE, A METHOD FOR PRODUCTION OF SUCH LIPASE AND A USE OF SUCH LIPASE

Number of Complete Specification: 570720

Term of Letters Patent: **Sixteen years commencing on** 4 September 1984

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Name of Convention Country in which basic application(s) filed: DENMARK

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Application number(s) of basic application(s): 4025/83

IN WITNESS whereof our Commissioner of Patents has caused these Our Letters Patent to be dated as of the 4 September 1984, and to be sealed with the seal of the Patent Office on 17 August 1988.

P.A. SMITH
Commissioner of Patents

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IMMOBILIZED LIPASE PREPARATION

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(56) **Prior Art Documents**

15163/83	550798	C11C 3/10, A23D 5/02, C12P 7/64
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(57) **Claim**

1. Method for production of an immobilized lipase preparation intended for interesterification of fats, wherein an aqueous solution of a microbial lipase is contacted with a particulate, macroporous, weak anion exchange resin which contains primary and/or secondary and/or tertiary amino groups and which exhibits a relatively large average particle size suitable for column operation without excessive pressure drop, at conditions, at which the lipase is bonded to the anion exchange resin during a period of time sufficient to bind the wanted amount of lipase to the anion exchange resin, whereafter the thus formed immobilized lipase is separated from the aqueous phase and the separated immobilized lipase is dried to a water content of between approximately 2 and 40%.

570720

FORM 10

SPRUSON & FERGUSON

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COMPLETE SPECIFICATION

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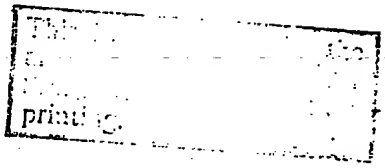

Priority:
Related Art:

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Complete Specification for the invention entitled:

"LIPASE, A METHOD FOR PRODUCTION OF SUCH LIPASE AND A USE OF SUCH LIPASE"

The following statement is a full description of this invention, including the best method of performing it known to us

SBR:JMC:0115F

Immobilized lipase preparations for transesterification of fats are known. Thus, in Danish patent application No. 563/77 (corresponding to US 4.275.081) an immobilized lipase preparation is described, whereby the lipase is produced by fermentation of species belonging to the genera Rhizopus, Geotrichum or Aspergillus, and whereby the lipase is attached on an indifferent particulate carrier which may be diatomaceous earth or alumina, and which exhibits a very high specific surface area. It has been deemed necessary to use an immobilized lipase preparation with very high specific surface area (i.e. small and porous carrier particles) in order to obtain the necessary high enzymatic activity. Interesterification can be carried out batchwise without a solvent with this immobilized lipase preparation; however, with this immobilized lipase preparation continuous interesterification in a column cannot be carried out on an industrial scale without the presence of a solvent, which has to be removed later, due to the above indicated fact that the preparation consists of small particles, which during column operation would generate an unacceptably high pressure drop. Also, a poster presented at Enz.Eng. 6, Kashikojima, Japan, 20 - 25 Sept. 1981 and the article in European Journal of Applied Microbiology and Biotechnology, No. 14, pages 1 - 5 (1982) indicates that an immobilized lipase preparation comprising lipase from Rhizopus delemar and a strong anion exchange resin (with quaternary amino groups) may be used for interesterification with n-hexane as a solvent. The enzyme recovery according to these references is very low, though. Also, from European patent application published with publication No. 0069599 an enzymatic

substantially without a solvent or other expensive auxiliary agents
expensive auxiliary agents in an economically feasible way.

Now, surprisingly according to the invention it has been found that a method for production of an immobilized lipase preparation, which is very easily performed, viz. by simple mixing of an aqueous solution of lipase and an ion exchange resin and which comprises a specific combination of a specified category of ion exchange resins and a specified proportion of water in the final immobilized lipase preparation opens up the possibility of carrying out the continuous interesterification without a solvent or other expensive auxiliary agents in an economically feasible way.

Thus, the method according to the invention for production of an immobilized lipase preparation intended for interesterification of fats is characterized by the fact that an aqueous solution of a microbial lipase is contacted with a particulate, macroporous, weak anion exchange resin which contains primary and/or secondary and/or tertiary amino groups and which exhibits a relatively large average particle size suitable for column operation without excessive pressure drop, at conditions, at which the lipase is bonded to the anion exchange resin during a period of time sufficient to bind the wanted amount of lipase to the anion exchange resin, whereafter the thus formed immobilized lipase is separated from the aqueous phase and the separated immobilized lipase is dried to a water content of between approximately 2 and 40%.

It is described generally in German published patent applications Nos. 2 905 671 and 2 805 950, Japanese published patent applications Nos. 54-76892 and 57-152886, US patent No. 4 170 696 and Chem. Abs. Vol 82, 27819d that

whereas hydrolysis of fats and synthesis of fats are less important applications according to the invention, which will be explained more detailed later in this specification, and in the second place it appears from the article that the activity yield is less than 1%, vide table 1 on page 109, as compared to an activity yield typically above 80% in relation to the immobilized lipase preparation prepared by means of the method according to the invention. This confirms our previous statement that immobilization of lipase is a very delicate problem.

It is intended that the expression "relatively large average particle size" is related to the average particle size of the product which is described in Danish patent application No. 563/77, and of which the majority of the particles have a particle size less than approximately 50 µm. It has been found that the temperature has no great effect on the activity yield, as it has been shown experimentally that the activity yield is virtually temperature independent in case the temperature during immobilization is kept between 5 and 35°C.

In order not to inactivate the enzyme the prior art interesterifications are carried out at relatively low temperature. This is made possible by the presence of the solvent, which is able to dissolve the fat, which might have a relatively high melting point. Surprisingly it has been found that the immobilized lipase preparation produced by means of the method according to the invention has a sufficient stability in the melted fat with a relatively higher temperature. Also, the pressure drop through the interesterification column loaded with the immobilized lipase

produced by means of the method according to the invention can be prepared with a high enzyme recovery which opens up the possibility for a cheaper continuous interesterification than the prior art interesterifications.

In a preferred embodiment of the method according to the invention the lipase is a thermostable lipase. Hereby a higher interesterification temperature is made possible, and thus a higher productivity. Furthermore by means of this embodiment it is possible to produce an immobilized lipase preparation which is well suited for interesterification of higher melting fats.

In a preferred embodiment of the method according to the invention the microbial lipase is derived from a thermophilic Mucor species, especially Mucor miehei. Mucor miehei is a good producer of 1,3-specific lipase, and thus a cheap product can be obtained.

In a preferred embodiment of the method according to the invention more than 90% of the particles of the macroporous weak anion exchange resin has a particle size between approximately 100 and 1000 µm, preferably between 200 and 400 µm. In this particle size interval a good compromise between high interesterification activity and low pressure drop is obtained.

In a preferred embodiment of the method according to the invention the proportion between the amount of the aqueous solution of the microbial lipase and the weight of weak anion exchange resin corresponds to 5,000 - 50,000 LU/g ion exchange resin (dry weight). In this manner sufficient lipase for the ion exchange resin is provided.

In a preferred embodiment of the method according to

reduction of enzyme activity may be observed due to the crosslinking agent it has been found that such treatment may raise the stability of the lipase preparation in aqueous media for a specific application. In relation to use of the immobilized lipase preparation as an interesterification agent there is no need for any improvement of the stability of the lipase preparation, as this stability is inherently excellent in the lipase preparation prepared according to the invention for this application. However, it has been found that the immobilized lipase preparation prepared in accordance with the method according to the invention advantageously can be used for hydrolysis of fats as well, and for this application an improvement of the stability of the lipase preparation is a desideratum, probably due to the combination of relatively high concentration of water and high temperatures in the reaction mixture, necessary for an industrially performed fat hydrolysis process.

Also, the invention comprises a use of the immobilized lipase preparation prepared by means of the method according to the invention, which is a method for interesterification of fats, wherein melted fats, ~~facultatively mixed with dissolved free fatty acid~~, is contacted with the immobilized lipase preparation prepared by means of the method according to the invention, without any solvent or other expensive auxiliary agents or substantially without any solvent or other expensive auxiliary agents. ~~The free fatty acids~~, with which the fats facultatively ~~may be~~ mixed according to the invention ~~are not~~ to be considered as expensive auxiliary ~~agents~~. By fats is meant either a pure triglyceride or a mixture of triglycerides from one or more sources.

$$OOO + P \rightleftharpoons POO + O$$

$$POO + P \rightleftharpoons POP + O,$$

where O = oleic acid, P = palmitic acid, and OOO, POO and POP are fats containing the indicated fatty acids in the order indicated, OOO thus being triolein.

250 mg of immobilized lipase preparation is mixed with 600 mg triolein (0.68 mmol) and 174 mg palmitic acid (0.68 mmol) dissolved in 12 ml petroleum spirit (temp. 80 - 100°C) in a 20ml glass tube with screw cap. The tubes are incubated in a water bath at 40°C and shaked for 1/2, 1 or 3 hours.

The reaction mixture is cooled, filtered and evaporated. The relative amount of OOO, POO and POP is determined by HPLC, and the percentage of incorporated P is calculated as

$$\% \text{ incorporated P} = \frac{\% POO + 2 \times \% POP}{3}$$

The equilibrium composition of the above indicated batch reaction mixture is approximately 43% POO and 10% POP or 21% incorporated P.

In some of the following examples the interesterification is carried out as a batch operation with or without solvent. By comparative tests it has been established that an immobilized lipase preparation, which has satisfactory interesterification activity and stability, as demonstrated by the batch interesterification test, and which possesses a particle size distribution and a physical strength required for satisfactory column operation, will operate satisfactorily by continuous operation in column as well, with or without solvent. Thus, a satisfactory batch test under these circumstances is evidence that a satisfactory

Then they were washed with plenty of water and drained on a Büchner funnel, drained weight approximately 16 g.

To each one of two 10 g portions was added a solution of 2.5 g Mucor miehei lipase (activity 93,000 LU/g) in 25 ml of water, and pH was adjusted to 6.0.

To the third portion was added a solution of 2.5 g of the above indicated Mucor miehei lipase in 50 ml of water, and pH was adjusted to 6.0.

The mixture was slowly agitated at room temperature (25°) for 2 hours. Hereafter the liquid was filtered off on a Büchner funnel.

One of the portions with 25 ml lipase solution was furthermore washed with 2 x 25 ml of water. The immobilized preparations were dried in vacuum.

For the interesterification assay 250 mg (dry weight) of the immobilized lipase preparations were moistened with 20 µl of water prior to mixing with the substrate.

Lipase Preparation Immobilized with	Interesterification, 1/2 hour %POO	%POP	%Incorporated P
2.5 g lipase in 25 ml without wash	25.8	6.85	13.2
2.5 g lipase in 25 ml with wash	30.1	7.65	15.1
2.5 g lipase in 50 ml without wash	26.8	6,86	13.5

This example demonstrates that subsequent water wash in order to remove unbound lipase is essential for obtaining a high interesterification activity, whereas the amount of water in which the lipase is dissolved during immobilization, is of minor importance.

Example 4

Two of the preparations from example 3 were reassayed with varying addition of water, i.e. the sample with 12.5 g lipase solution and that with 25 g lipase solution, both with a reaction time of 2 hours. The effect of the moisture content on the interesterification activity appears from the following table.

Sample	µl water added to 250 mg dry weight	Estimated moisture in sample %	Interesterification, 1/2hour %POO	%POP	%incorporated P
12.5 g	0	2.6	18.2	2.27	7.6
	20	9.6	25.6	6.55	12.9
	50	18.5	23.4	5.85	11.7
	100	29.9	15.3	3.84	7.6
25 g	0	3.0	19.1	2.04	7.7
	20	10.0	28.6	7.65	14.6
	50	18.8	25.4	5.25	12.0
	100	30.1	18.6	4.55	9.2

This example shows that the optimal moisture content is approximately 10%.

Example 5

One of the preparations from example 3 was reassayed with varying amounts of added water. The sample with 25 g lipase solution and 4 hours reaction time was used.

Sieve fraction	Interesterification, 1/2 hour: Crushing prior to immobilization			Crushing after immobilization		
	%POO	%POP	%incorp.P	%POO	%POP	%incorp.P
180–300 µm	30.1	7.78	15.2	25.7	6.39	12.8
425–500 µm	25.7	6.66	13.0	21.7	5.50	10.9
600–710 µm	19.2	5.06	9.8	17.2	4.38	8.7
850–1000µm	12.7	3.22	6.4	14.3	3.90	7.4

It clearly appears that it is an advantage to use the fine sieve fractions to obtain maximum interesterification activity, but the need for a low column pressure drop makes a compromise necessary.

Example 7

This example illustrates the effect of different categories of macroporous weak anion exchange resins (type of matrix, functional groups, particle size) on the batch interesterification activity of the immobilized lipase preparation

In the case of Duolite ES 562, Duolite A 561, Duolite A 7, Amberlite IRA 93, and Amberlyst A 21 4.25 grams dry weight resin was washed with water, mixed with 1 gram of Mucor miehei lipase (93,000 LU/g) in 20 ml of water, the mixture being adjusted to pH 6.0, and rotated slowly for 2 hours at room temperature. After filtration, each preparation was washed with 250 ml of water. In the case of Duolite A 378 8.5 grams was mixed with 2 grams of lipase and finally washed with 250 ml of water. All were dried in vacuum at room temperature. In the case of Duolite A 365, Duolite S 587, and Dowex MWA-1 4.25 gram dry weight resin was mixed with 1 gram of Mucor miehei lipase (124,000 LU/g) in approximately 10 ml of water for 2 hours by rotation at room

Anion exchange resin	Matrix	Funct. groups	Partc. sizes, µm (>85%)	Water %	Batch activity, % POO	% POP	1/2 hour incorp. % P
Duolite ES 562	Phenol-formalde-hyde	Tert. amine	212 - 425	13.8	26.7	6.8	13.4
Duolite A 561	Phenol-formalde-hyde	Tert. amine	300 - 1200	13.0	14.8	3.2	7.1
Duolite A 7	Phenol-formalde-hyde	Second. amine	300 - 1200	13.5	9.5	2.5	4.8
Duolite A 378	Polysty-renic	Tert. amine	300 - 1100	6.3*	14.3	3.3	7.0
Amberlite IRA 93	Styrene-DVB	Poly-amine	400 - 500	12.2	10.8	2.9	5.5
Amberlyst A 21	Styrene-DVB	Tert. amine	425 - 850	11.1	10.6	2.7	5.3
Duolite A 365	Polysty-renic	Prim. amine	300 - 1200	11.5	15.5	3.7	7.6
Duolite S 587	Phenol-form.	Amines	300 - 1100	7.4	25.4	6.4	12.7
Lewatit MP 62	Polysty-renic	Amines	300 - 1200	13.6	16.9	3.9	8.2
DOWEX MWA-1	Styrene-DVB	Tert. amine	300 - 1200	10.5	21.0	4.9	10.3

* 5% water was added before batch assay

Example 8

30 g Duolite ion exchange resin type ES 562 was suspended in approximately 75 ml of H_2O, and pH was adjusted to 6.0 with 4 N NaOH. The ion exchange resin was washed with water on a suction filter, and excess of water was sucked away. The wet ion exchange resin (approximately 45 g) was divided in three equal portions.

The first third was mixed with a solution of 1 g Mucor miehei lipase (210,000 LU/g) in 20 ml of H_2O adjusted to pH 6.0. After mixing the pH was readjusted to 6.0, and the mixture was allowed to react for 4 hours at 5°C with magnetic stirring. During this period the pH dropped to 5.45. The mixture was transformed to a Buchner funnel with a few milliliters of water and as much as possible of the solution was sucked away (14 ml). The resin was further dried in vacuum to a water content of 10.0%. Yield 8.27 g.

1) demineralized water
2) 0.05 M sodium phosphate, pH 6.0
3) 0.5 M - - , pH 6.0
4) 0.05 M - chloride
5) 0.5 M - -

Other five 5.25 gram portions (dry weight 4.25 g) of Duolite ES 562 ion exchange resin were equilibrated with 20 ml of 1) - 5) above. After decantation, the corresponding lipase solutions were added to the wet ion exchange resin particles adjusted to pH 6.0, and the containers were rotated slowly over 2 hours at 25°C. The preparations were then collected by filtration and each washed with 250 ml demineralized water followed by drying in vacuum at 25°C (64 hours). The results of the interesterification activity assay are shown below:

Salt/concentration	Yield (g)	% H_2O *	Interesterification activity, 1/2 hour % POO	% POP	% incorporated P
No salt	4.51	4.7	23.1	5.7	11.5
0.05 M phosphate	4.48	5.3	21.9	5.3	10.8
0.5 M -	4.57	4.6	20.3	5.1	10.2
0.05 M NaCl	4.54	4.6	23.4	5.7	11.6
0.5 M -	4.43	4.9	19.2	4.6	9.5

* additional H_2O up to a total of 10% was added before assay.

Example 10

This example shows the effects of high concentrations of sodium acetate during lipase immobilization on the interesterification activity of the preparations.

Five 1.00 g portions of Mucor miehei lipase, diafiltrated and freeze-dried, 93,000 LU/g, were separately dissolved in 20 ml of the following liquids:

The activity left in the total filtrate corresponded to 18% of the original activity.

Aspergillus niger esterase was obtained by ultrafiltration of the commercial product Palatase from NOVO. 15 ml PALATASE of 2790 LU/ml was immobilized on 4.25 g of ES 562 as described above whereby 4.77 g immobilized preparation with 7.6% water was obtained. The filtrate contained 13% of the original LU-activity.

Candida cylindracea lipase from Amano (type OF) was similarly immobilized by mixing 4.25 g of ES 562 with 1.40 g Amano lipase OF in 15 ml of water, pH 6.0. The yield was 4.62 immobilized preparation with 6.5% of water and 0.2% activity remaining in the filtrate.

The three preparations were characterized as follows:

1) By the standard batch assay at 40°C
2) By a triolein (000)/decanoic acid (D) batch interesterification without solvent at 60°C using 3.00 g 000, 0.600 g D, and 250 mg dry lipase preparation hydrated to approximately 10% water.

For comparison purposes also results for a Mucor miehei lipase preparation, as described in example 13, are listed as well:

other expensive auxiliary agents, as described in the following example 12.

Example 12

This example illustrates continuous interesterification of fats without solvent or other expensive auxiliary agents, using an immobilized lipase preparation prepared by means of the method according to the invention in a packed bed reactor.

Immobilization

2.20 grams of Mucor miehei lipase (81,000 LU/g) was dissolved in 20 ml of water, mixed with 10 grams washed (8.5 g dry weight) Duolite ES 562 ion exchange resin with more than 80% of the particles between 200 and 400 µm. The mixture was adjusted to pH 5.0, and left for 4 hours at 5°C with magnetic stirring. After filtration and wash with a small amount of water the preparation was dried in vacuum at room temperature. The yield was 9.05 grams, containing 9.3% water. The activity remaining in the filtrate was 8% of the total, initial amount. The batch interesterification activity was 30.6% POO, 7.7% POP at ½ hour or 15.3% incorporated P.

Test in column

2 grams of this immobilized lipase preparation was placed in a column and a solvent-free substrate consisting of olive oil/palmitic acid in the ratio 2.5:1 w/w was continuously fed through at 60°C. The performance of the lipase preparation is shown in the table below.

Sample/time	Flow gTG/h/ g enz.	Composition (HPLC) OOO %	POO %	POP %	Conversion x, % (GLC)
Olive oil/ start	-	42.3	22.5	3.8	0
17 hours	5.7	30.5	30.1	11.6	-
208 1/2 hours	2.5	33.8	28.8	8.6	28
233 -	0.61	22.2	34.8	16.5	67
475 -	1.8	35.1	28.8	8.7	28
Equilibrium (batch)	-	17.4	36.0	20.6	100

18 l of Mucor miehei lipase (10,100 LU/ml) adjusted to pH 6.0 was recirculated at 30 l/h for 6 hours with pH control. After displacement with 20 l of water a combined volume of 37 l contained 126 LU/ml corresponding to 97% immobilization yield. The column was further washed with another 20 l of water and the preparation was vacuum dried at room temperature whereby 6.0 kg (97% dry matter) immobilized lipase preparation was obtained. The batch interesterification activity was 30.2% POO, 6.9% POP at 1/2 hour or 14.7% P_{inc}.

Application experiment No. 1

4.0 g of the immobilized lipase preparation was filled into a water jacketed column with an internal diameter of 1.5 cm. The temperature in the column was maintained at 60°C. An olive oil/decanoic acid substrate with a composition of 2.5/1 (w/w) was pumped through a precolumn containing 30 g Duolite S 561 saturated with 21 ml of ion exchanged water and further through the main column containing the immobilized lipase preparation. The flow rate was controlled in order to keep the composition of the output at a value corresponding to approximately 65% conversion, i.e. 23% DOO in the final triglyceride (DOO means a triglyceride with one decanoic acid unit (in outer position) and two oleic acid units).

On the assumption that the decrease of the activity of the immobilized lipase follow a first order reaction the half life can be estimated to 3200 hours. With an initial activity of 2.4 g triglyceride/hour/g enzyme preparation the productivity is appromately 8.3 tons of triglyceride /kg enzyme preparation assuming a run time of two half lives. In fig. 1 the logarithm to the flow rate is plotted against the time

of 1.5 parts of beef tallow and 1 part of soy bean oil which were mixed at 70°C. BHT antioxidant was added in a concentration of 0.1%. To characterize the individual components and to follow the interesterification reaction, HPLC was used to analyze the triglyceride composition of the sustrate components, the initial mixture and the interesterified mixture. An initial batch reaction with 2.75 grams immobilized Mucor miehei lipase preparation, 24 grams tallow, and 16 grams soy bean oil was run 16.5 hours at 65°C. HPLC showed that the ratio of LPO- to LLL-triglyceride (L: Linoleic, P: Palmitic, O: Oleic) in the mixture increased from 0.62 to 1.16, this latter figure presumably being close to the equilibrium ratio.

Melting Properties of the Interesterified Mixture

The change in melting properties due to interesterification was analyzed by dilatation according to the official IUPAC-method (IUPAC: standard methods for the analysis of oil, fats, and derivatives, 6th ed., method No. 2.141 (1979)). The results appear from the table below with a

hydrolysis.

To a 1:1 mixture of pure beef tallow and water, 40 grams of each, was added immobilized lipase, prepared as described in example 13, in an amount corresponding to 100 LU/g fat, or 0.13 grams of immobilized lipase preparation, assuming a load of 30.000 LU/g of immobilized lipase. An effective mixing was obtained by magnetic stirring at 48°C. The initial pH value in the water phase was adjusted to 8.0. After 4 days the enzyme was separated and the fat phase analyzed for degree of hydrolysis (DH). Duplicate tests were carried out. The recovered immobilized enzyme was used for a 2' run and a comparison experiment with soluble lipase, also added in an amount corresponding to 100 LU/g fat, was carried out. In this case the aqueous phase was used for the 2' run. Three tests were carried out with soluble lipase. The results were as follows:

Preparation	Sample No.	%DH, 4 days, 48°C 1' run	2' run
Immobilized lipase	1	30	31
	2	35	37
Soluble lipase	1	61	5
	2	55	8
	3	46	7

The pH value dropped to about 6.8 with the immobilized lipase and to about 6.3 in the 1' run and to about 7.4 in the 2' run with the soluble lipase. %DH was calculated as the acid value (AV) divided by the saponification value (SV).

during contact between ion exchange resin and aqueous solution is between 5 and 7.

7. Method according to claim 1 to 6, wherein the contact time is between 0.5 and 8 hours.

8. Method according to claim 1 to 7, wherein the separation is performed by simple filtration.

9. Method according to claim 1 - 8, wherein the drying is carried out to a water content between 5 and 20%.

10. Method according to claims 1 - 9, wherein the particulate, macroporous, weak anion exchange resin is brought together with an aqueous solution of a crosslinking agent, preferably an aqueous glutaraldehyde solution in a concentration between 0.1 and 1.0% w/w, before, during or after the contact between the particulate, macroporous weak anion exchange resin and the aqueous solution of the microbial lipase, whereafter the remaining solution of cross-linking agent is separated.

11. Method for interesterification of fats, wherein melted fats, ~~facultatively mixed with melted free fatty-acid~~, is contacted with the immobilized lipase preparation produced according to claim 1 to 10 without any solvent or other expensive auxiliary agents or substantially without any solvents or other expensive auxiliary agents.

12. Method for hydrolysis of fats, wherein an emulsion of triglyceride and water is contacted with the immobilized lipase preparation produced according to claims 1-10 without any solvent or other expensive auxiliary agents or substantially without any solvents or other expensive auxiliary agents.

~~13. Method for synthesis of fats, wherein a mixture~~

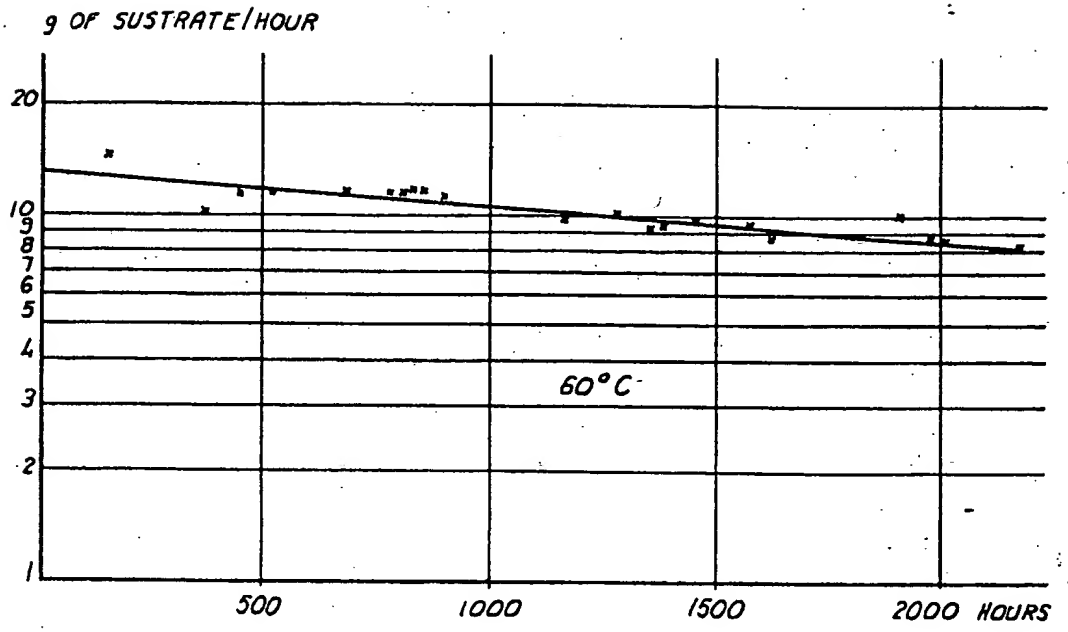
g OF SUSTRATE/HOUR
20
10
9
8
7
6
5
4
3
2
1
60° C
500
1000
1500
2000 HOURS

FIG. 1.

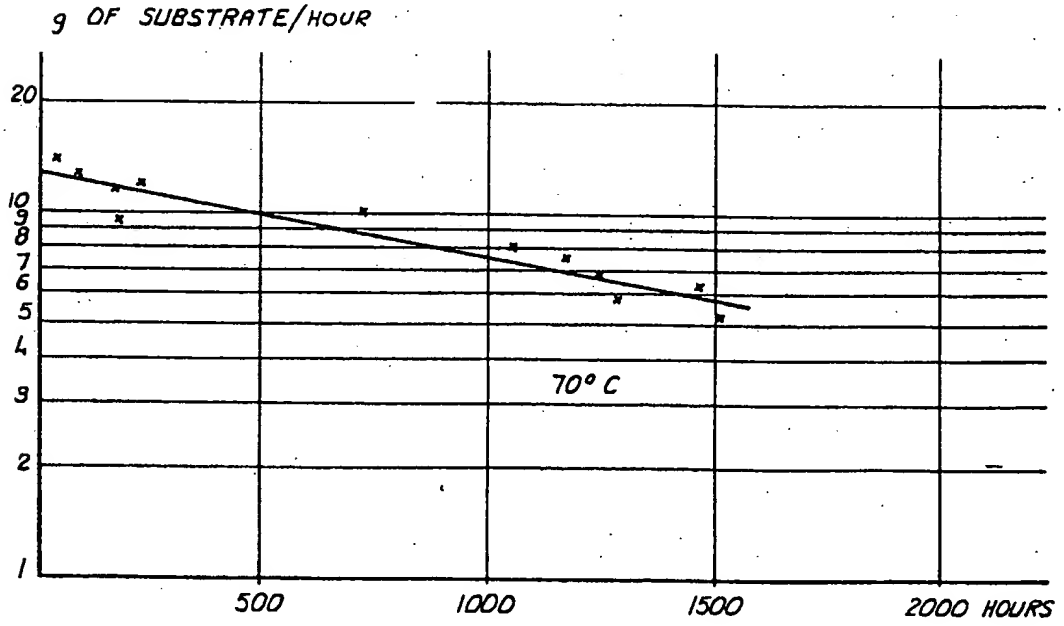
g OF SUBSTRATE/HOUR
20
10
9
8
7
6
5
4
3
2
1
70° C
500
1000
1500
2000 HOURS

FIG. 2.